



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/12, 31/07</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/37874</b> <b>(43) International Publication Date:</b> 3 September 1998 (03.09.98)
<b>(21) International Application Number:</b> PCT/EP98/00628 <b>(22) International Filing Date:</b> 5 February 1998 (05.02.98) <b>(30) Priority Data:</b> 9700708-2 27 February 1997 (27.02.97) SE <b>(71) Applicant (for all designated States except US):</b> ASTAC- AROTENE AB [SE/SE]; Idrottsvägen 4, S-134 40 Gustavs- berg (SE). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> WADSTRÖM, Torkel [SE/SE]; Rektorsvägen 7, S-224 67 Lund (SE). ALEJUNG, Pär [SE/SE]; Järnåkravägen 25 A, S-222 25 Lund (SE). <b>(74) Agents:</b> NILSSON, Brita et al.; AB Stockholms Patent- byråZacco & Bruhn, P.O. Box 23101, S-104 35 Stockholm (SE).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title:</b> ORAL PREPARATION FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF <i>HELICOBACTER SP.</i> INFECTION  <b>(57) Abstract</b> <p>An oral preparation for the prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract (especially the human stomach) caused by <i>Helicobacter sp.</i> (especially <i>H. pylori</i>) infection is described. The preparation comprises a prophylactically and/or therapeutically effective amount of at least one type of xanthophylls. The most preferred xanthophyll is astaxanthin which is soluble in oil, preferably naturally produced astaxanthin which is esterified with fatty acids. The oral preparation may further comprise carbohydrate structures, such as those which derive from the cell wall of the production alga <i>Haematococcus sp.</i>. The preparation may also comprise a prophylactically and/or therapeutically effective amount of a water soluble antioxidant, such as ascorbic acid (vitamin C). The oral preparation is presented in a separate unit dose or in mixture with food.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## ORAL PREPARATION FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF *HELICOBACTER SP.* INFECTION

5 The present invention relates to an oral preparation for the prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract caused by *Helicobacter sp.* infection. The preparation comprises at least one type of xanthophylles, preferably naturally produced  
10 astaxanthin.

### **Background of the invention**

15 Since a few years *Helicobacter pylori* is classified as a primary cause of type B gastritis in humans.

Various *Helicobacter sp.* infect different animals, and must penetrate the gastric surface mucous layer [O'Toole PW et al., Mol Microbiol 1994, 14:691-703] to colonize the gastric epithelium and sub-mucosa [Wadström T et al., Aliment Pharmacol Ther 1996, 10(suppl 1): 17-28.; Valkonen KH et al., Infect immun 1994, 62:3640-3648.; Moran AP et al., J Appl Bacteriol 1993, 75:184-189.;  
20 Wadström T et al., Eur J Gastroenterol Hepatol 1993, 5 (suppl 2):512- 515].

*Helicobacter* is a flagellated motile organism probably penetrating the gastric mucous layer rapidly and efficiently with spiral movements associated with the unique spiral shape of this pathogen (*Helicobacter* from *helix*, which is Latin for  
25 spiral).

*Helicobacter pylori* can cause drastic changes of the gastric mucous membrane barrier functions in an early infection (i.e. type B gastritis) with breakdown of the hydrophobic lining of the gastric epithelium. This can cause back-flow of acid  
30 and pepsin from the lumen into the mucosa to cause peptic ulcers in the stomach and duodenum. It seems likely that this breakdown of the mucosa

barrier also affects the uptake in the gastric mucosa of a number of substances in food such as certain food-associated carcinogens.

5 Xanthophylles, including astaxanthin, is a large group of carotenoids containing oxygen in the molecule in addition to carbon and hydrogen. The carotenoids are produced *de novo* by plants, fungi and some bacteria [Johnson E.A. and Schroeder W.A., 1995, Adv In Biochem Engin. Biotechn. 53: 119-178]. In biological tests astaxanthin has been shown to possess clearly the best  
10 antioxidative properties compared to other carotenoids [Miki W., 1991, Pure and Appl Chem 63 (1) : 141-146].

At present, the therapeutic treatment of inflammation in the mucous membranes of mammalian gastrointestinal tract caused by *Helicobacter sp.* infection, mainly involves the use of so-called proton pump inhibitors, such as Losec®  
15 (omeprazol), and in case of gastric ulcers different antibiotics (which may cause the development of resistant strains).

### Description of the invention

20 The present invention provides an oral preparation for the prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract caused by *Helicobacter sp.* infection, which comprises a prophylactically and/or therapeutically effective amount of at least one type of xanthophylles.

25

The oral preparation according to the invention may comprise a mixture of different types of xanthophylles or different forms of the same xanthophyll, such as a mixture of synthetic astaxanthin and naturally produced astaxanthin.

30 In a particular embodiment of the invention the mammalian gastrointestinal tract is the human stomach, and the *Helicobacter sp.* is *H. pylori*.

The mechanism of the prophylactic and therapeutic effect of the xanthophylls in the treatment of inflammation in the mucous membrane of the mammalian gastrointestinal tract caused by *Helicobacter sp.* infection is not known, but it is believed that the antioxidative properties of the xanthophylls, which are soluble  
5 in fat/oil, play an important role in the protection of the hydrophobic lining of the mucous membrane so that *Helicobacter sp.* cannot colonize.

In a preferred embodiment of the invention, the xanthophyll is dissolved in an oil of food grade.

10

In another preferred embodiment the type of xanthophyll is astaxanthin, particularly astaxanthin in a form esterified with fatty acids.

In yet another preferred embodiment the astaxanthin derives from a natural  
15 source, particularly a culture of the alga *Haematococcus sp.* [Renström B. et al, 1981, Phytochem 20(11) :2561-2564].

The oral preparation according to the invention may further comprise carbohydrate structures, such as lipopolysaccharides, polysaccharides and  
20 glycoproteins.

At present, the most preferred embodiment of the invention comprises algal meal having astaxanthin in esterified form with fatty acids dissolved in small droplets of naturally occurring oil and naturally occurring carbohydrate  
25 structures in the partially disrupted cell walls.

The oral preparation of the invention may comprise additional ingredients which are pharmacologically acceptable inactive or active in prophylactic and/or therapeutic use, such as flavoring agents, and a prophylactically and/or  
30 therapeutically effective amount of a water soluble antioxidant, especially ascorbic acid (vitamin C).

The oral preparation is presented in a separate unit dose or in mixture with food. Examples of separate unit doses are tablets, gelatin capsules and predetermined amounts of solutions, e. g. oil solutions, or emulsions, e.g. water-in- oil or oil-in-water emulsions. Examples of foods in which the preparation of the invention may be incorporated is dairy products, such as yoghurt, chocolate and cereals.

Another aspect of the invention is directed to a method of prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract caused by *Helicobacter sp.* infection, which comprises administration to said mammal of an oral preparation according to the invention.

The daily dosis of the active ingredient of the invention will normally be in the range of 0.01 to 10 mg per kg body weight for a human calculated on the amount of astaxanthin, but the actual dosis will depend on the mammalian species and the individual species-specific biological effect.

### Experiments

The oral preparation used in the experiments is the xanthophyll astaxanthin which is commercially produced via culturing of the algae *Haematococcus sp.* by AstaCarotene AB, Gustavsberg, Sweden.

Astaxanthin from other sources, and other xanthophylles as well, are expected to be similarly useful for the purposes of the invention. An advantage of using astaxanthin from algae is, however, that the astaxanthin exists in a form esterified with fatty acids [Renström B. et al, *ibid*], which esterified astaxanthin thereby is more stable during handling and storage than free astaxanthin.

The naturally produced astaxanthin can be obtained also from fungi and crustaceans, in addition to from algae [Johnson E.A. and Schroeder W.A., *ibid*].

Fifty 6 - 8 weeks old Balb/cA mice weighing 28- 30 g were infected with *H. pylori* by administration of  $10^8$  cfu in phosphate buffer through a gastric tube into the stomach. The treatment was repeated three times on one-day intervals [Aleljung P., et al., 1996, FEMS Immunol Med Microbiol. 13: 303-309].

5

After 14 days, 10 mice were sacrificed and cultures were made on stomach biopsies to isolate *H. pylori*. The culturing takes seven days.

10

Twenty-one days after the infection with *H. pylori* half of the remaining animals were given feed supplemented by algal meal corresponding to 0.3 mg astaxanthin per animal per day for a period of 10 days.

15

On day 30 half of the animals in each group were sacrificed and culturing was made in a similar way as disclosed above.

On day 40 the rest of the animals were sacrificed and culturing was made in a similar way as disclosed above.

20

The results are given in Table 1.

**Table 1.**

The number of animals positive for *H. pylori* per number of sacrificed animals.

25

30

Day	Treated animals	Control animals
14	—	8 / 10
30	0 / 8	8 / 10
40	0 / 10	7 / 10

From the results in Table 1 it is evident that the algal meal containing astaxanthin has a therapeutic effect and can be used for prophylactic purposes.

## CLAIMS

5

1. Oral preparation for the prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract caused by a *Helicobacter sp.* infection, which comprises a prophylactically and/or therapeutically effective amount of at least one type of xanthophylls.

10

2. Oral preparation according to claim 1, wherein the mammalian gastrointestinal tract is the human stomach and the *Helicobacter sp.* is *H. pylori*.

15

3. Oral preparation according to claim 1 or 2, wherein the xanthophyll is dissolved in an oil of food grade.

4. Oral preparation according to any one of claims 1-3, wherein the type of xanthophyll is astaxanthin.

20

5. Oral preparation according to claim 4, wherein the astaxanthin is in a form esterified with fatty acids.

25

6. Oral preparation according to claim 4 or 5, wherein the astaxanthin derives from a natural source.

7. Oral preparation according to claim 6, wherein the natural source is a culture of the alga *Haematococcus sp.*

30

8. Oral preparation according to any one of the claims 1-7, which further comprises carbohydrate structures.



9. Oral preparation according to claims 7 and 8, which comprises algal meal having astaxanthin in esterified form with fatty acids dissolved in small droplets of naturally occurring oil and naturally occurring carbohydrate structures in the partially disrupted algal cell walls.

10. Oral preparation according to any one of claims 1 - 9, which further comprises a prophylactically and/or therapeutically effective amount of a water soluble antioxidant.

11. Oral preparation according to claim 10, wherein the water soluble antioxidant is ascorbic acid (vitamin C).

12. Oral preparation according to any one of claims 1 - 11, which is presented in a separate unit dose or in mixture with food.

13. Method of prophylactic and/or therapeutic treatment of inflammation in the mucous membrane of mammalian gastrointestinal tract caused by *Helicobacter sp.* infection, which comprises administration to said mammal of an oral preparation according to any one of claims 1 - 12.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00628

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/12 A61K31/07

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9524 Derwent Publications Ltd., London, GB; AN 95-182023 XP002067980 & JP 07 099 924 A (NIPPON SUISAN KAISHA LTD) , 18 April 1995 see abstract ---	1,3,4,6, 12
X	DATABASE WPI Week 9603 Derwent Publications Ltd., London, GB; AN 96-026969 XP002067981 & JP 07 300 421 AA (ITANO REITO KK) , 14 November 1995 see abstract --- -/-	1,4-6,12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

15 June 1998

Date of mailing of the international search report

15. 07. 1998

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Alvarez Alvarez, C

## INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/EP 98/00628

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00130 A (THE HOWARD FOUNDATION ET AL.) 5 January 1995 see page 6, line 1 - line 5 see page 7, line 15 see page 8, line 1 - line 8 see page 8, line 15 see examples 7,9-12	1,3-5,8, 10-12
Y	---	2,6,7,13
Y	P. BUBRICK: "Production of astaxanthin from Haematococcus" BIORESOURCE TECHNOLOGY, vol. 38, 1991, pages 237-239, XP002067978 see the whole document	6,7
Y	---	2,13
Y	D. BAGCHI ET AL.: "Production of reactive oxygen species by gastric cells in association with Helicobacter Pylori" FREE REDICAL RESEARCH, vol. 24, no. 6, 1996, AMSTERDAM, NL, pages 439-450, XP002067979 see Discussion	2,13
X	---	1,4,12
X	WO 96 23489 A (BASF AG ET AL.) 8 August 1996 see page 3, line 14 - line 38 & GB 2 280 110 A -----	1,4,12

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 98/00628

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claim 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No

PCT/EP 98/00628

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9500130 A	05-01-1995	AU 7005694 A GB 2280110 A,B ZA 9404633 A	17-01-1995 25-01-1995 25-10-1995
WO 9623489 A	08-08-1996	DE 19503604 A DE 19539743 A AU 4715796 A CA 2210957 A EP 0806946 A	08-08-1996 30-04-1997 21-08-1996 08-08-1996 19-11-1997

8

**THIS PAGE BLANK (USPTO)**